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CULTURES OF A LEPTOTHRIX FROM A CASE OF PARINAUD'S CONJUNCTIVITIS

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This form of chronic conjunctivitis is characterized by the unilateral development of nodules beneath the conjunctiva, with involvement of the corresponding preauricular gland. Its etiology has been obscure since the first description by Parinaud in 1889. We have not reviewed all the available literature. However, Karl Hoor¹ has reviewed 43 cases and reported one of his own; and F. H. Verhoeff and G. S. Derby² have reviewed 22 cases in addition to one of their own.

In many cases the inoculations of guinea-pigs and rabbits have proved the absence of tubercle bacilli. In the cases in which tubercle bacilli have been demonstrated it seems probable that they either concurrently or secondarily accompanied the true etiologic agent.

In 1913, Verhoeff³ made a notable contribution. In 11 of 12 consecutive cases examined histologically he found, in addition to the same characteristic histologic picture, a minute filamentous organism. The filaments were present in irregular masses, from 10-60 μ in diameter, near or within the areas of cell necrosis which are especially prominent just beneath the epithelium. They can also be found in the superficial lymph spaces and this, according to Verhoeff, explains the early involvement of the regional glands.

The individual filaments were extremely delicate, about the diameter of the influenza bacillus; and varied from a few microns to 30 microns in length. They were straight, or more often irregularly curved or bent. They did not stain readily and were best demonstrated by a modified Gram's stain when the filaments exhibited rather regularly distributed granules. These granules varied in size and were "never exactly centered in the axis of the filament but project noticeably above its surface." No branching of the filaments was observed. Verhoeff suggested that it might be provisionally classed as a leptothrix.

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¹ Monatsbl. f. Augenh., 1906, 44, p. 289.

² Arch. Ophth., 1904, 33, p. 389.

³ Arch. Ophth., 1913, 42, p. 345.

We believe we have cultivated this leptothrix but do not insist on its identity with that described by Verhoeff as our organism does not exhibit the granular form.

REPORT OF CASE

History.—H. H., white, boy, aged 14 years, was admitted to the Cincinnati General Hospital, April 4, 1917; discharged cured, May 21, 1917. Did not feel well for a period of ten days before admission. The right eye began to inflame together with a swelling of the preauricular glands on the same side. There was no history of having played with any domestic animals nor had there been any illness in the family. On admission the eye showed decided ptosis with evidence of considerable conjunctivitis. There were no symptoms involving the cornea or iris. On everting the lids large nodular masses were found in the upper and lower lids. The preauricular glands were enlarged forming a mass about $1\frac{1}{2}$ by 1 inch and elevated about $\frac{1}{2}$ inch above the surrounding tissues (Fig. 1). During 25 days after admission the patient had an irregular fever ranging from 97-100 F. The treatment of the lids consisted in daily applications of 1% silver nitrate and the instillation of a 25% argyrol solution.

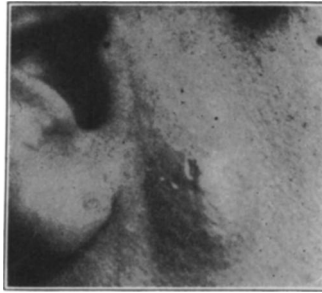


Fig. 1.—The preauricular gland some time after it had been opened.

Bacteriologic Examination.—Smears and cultures made at the time of admission from the mucopurulent secretion on the conjunctiva yielded nothing excepting 2 colonies of a diphtheroid bacillus. A 250 gm. guinea-pig inoculated intraperitoneally with the same material remained well during 20 days. When killed it was found to be normal.

Smears and cultures were made from pus collected from the preauricular gland 8, 10, and 17 days after admission. The smears were fixed by heat and by methyl alcohol and were stained with Loeffler's methylene blue, Bordet's carbol toluidin blue, Nicolle's carbol gentian violet, anilin Hoffman's violet, the tubercle method, and Giemsa without revealing any micro-organisms.*

The cultures were made on + 0.5 agar slants containing dextrose or maltose, with and without human blood; glycerol agar with rabbit blood; oiled broth containing ascites fluid and sterile guinea-pig kidney, and Dorset's egg medium.

* Prolonged staining in dilute warm Giemsa's stain was not used. This method has twice revealed bacteria which were not found by any of the ordinary methods of staining—once enormous numbers of filaments containing metachromatic granules and resembling *B. fusiformis*, apparently in pure culture in an abscess of the pancreas; and again a delicate filamentous organism in the pus of a case of hand infection and in the pus from the secondary meningitis which developed in this case.

They were incubated under aerobic, partial tension and anaerobic conditions at 37 C. and watched for about a month before being discarded as sterile. Following the isolation of the leptothrix, described below from Mouse 1, on coagulated egg yolk, pus collected from the preauricular gland 17 days after the patient was admitted was planted on this medium and incubated under anaerobic and partial tension conditions. These cultures were not examined for 2 weeks when the partial tension slants showed some very fine colonies of minute polymorphous rods resembling those found in the abscesses in Mouse 1. Only once was another organism encountered in the cultures and this was an actively motile vibrio which appeared on a single slant out of a considerable number inoculated from the first specimen of pus examined. As it grew readily on all mediums and was not encountered again it was discarded as a contamination.

Animal Inoculations.—The first specimen of pus aspirated from the preauricular gland (8 days after admission) was inoculated in the form of a

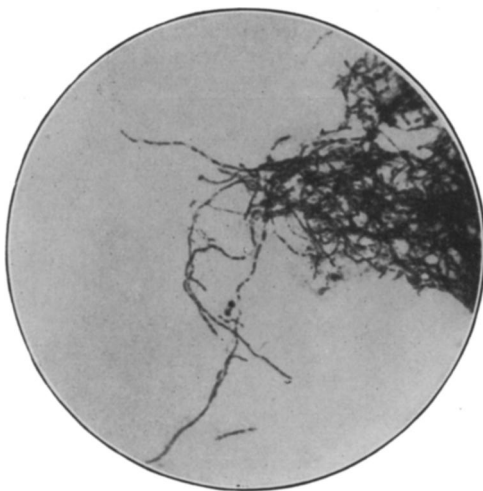


Fig. 2.—The irregularly staining leptothrix isolated on egg yolk from the muscular abscesses of Mouse 1. $\times 1000$.

densely clouded salt solution suspension into the subcutis of a rabbit, guinea-pig, and white mouse. The rabbit and guinea-pig developed slight local reactions which were completely healed in 14 days.

White Mouse 1: Two days after inoculation there was present a somewhat vesicular swelling about 4 mm. in diameter at the site of inoculation. Two days later the local swelling had subsided to about 2 mm. but now the left eye appeared to be suppurating. By the 6th day both eyes were closed and exuded a considerable amount of pus. Smears showed chiefly cocci and solid staining diphtheroid bacilli of the d^2 type. On the 9th day pus from one of the eyes was placed on the conjunctiva of Mouse 2, which remained well during a months' observation. On the 21st day, Mouse 1 died. The spleen and liver were congested and near the tendinous insertions of both knee joints there were muscular abscesses which microscopically showed numerous minute polymorphous gram-negative rods.

The other organs and heart blood showed no bacteria. The bacteria in the muscular abscesses stained poorly with Loeffler's blue or carbol toluidin blue and best after steaming hot Giemsa solution. The abscesses contained a caseous pus which was cultured on a variety of mediums under aerobic, partial tension and anaerobic conditions. No growth appeared in any of the cultures excepting that on a slant of coagulated egg yolk kept under anaerobic conditions and one kept at partial tension, there appeared in 48 hours 30 or 40 colonies, about 0.25 mm. in diameter, raised, dryish, and unpigmented. Microscopically these showed the presence of very delicate rods and filaments (Fig. 2). Attempts to transplant this leptothrix to any of the ordinary mediums failed.

Some of the pus from Mouse 1 injected into Mouse 5 seemed to reproduce the infection.

White Mouse 3: Pus collected on the 17th day from the preauricular gland was rubbed into the left eye. It remained normal for 27 days. The animal was then killed and found to be normal.

White Mouse 4: The animal was inoculated subcutaneously with the same pus used for Mouse 3 and remained well for 27 days. After the animal had been killed examination revealed it to be normal.



Fig. 3.—Mouse 5 inoculated subcutaneously with the abscess pus from Mouse. 1.

White Mouse 5: A mixture of the pus from the eyes and muscular abscess of Mouse 1 was injected subcutaneously. It appeared well and showed no signs of local reaction for 3 days. On the 4th day it appeared to have photophobia and on the 6th day its eyes were closed with double purulent conjunctivitis. It was photographed on the 7th day (Fig. 3). The eyes were still purulent on the 14th day but by the 18th day they were well. The mouse was killed and found free from muscular or other lesions.

Owing to a lack of white mice the following inoculations only could be performed with the culture isolated. A very young white rat, an adult white rat and a single wild mouse (*Mus musculus*) were inoculated subcutaneously with emulsions of the anaerobic egg yolk culture. They showed no symptoms during 2 weeks observation and when killed were found normal. The egg yolk cultures died after being kept at room temperature for 3 months.

SUMMARY AND CONCLUSIONS

Pus aspirated from the preauricular gland of a case of Parinaud's conjunctivitis and injected subcutaneously in a white mouse, produced an infection, after an incubation period of from 4-5 days, characterized

by purulent conjunctivitis and death. From muscular abscesses found postmortem, a leptothrix was isolated which grew only on slants of egg yolk incubated under partial tension and anaerobic condition. Pus from the mouse injected into a second white mouse reproduced the symptoms but resulted in recovery. The leptothrix isolated was non-pathogenic for white rats and a wild mouse. White mice were not inoculated with the pure culture. Later in the course of the human case a similar organism was isolated on egg yolk directly from the pre-auricular gland. It seems probable, though not certain, that the organism grown by us is identical with that found in sections by Verhoeff. The use of a guinea-pig and a rabbit seemed to rule out the presence of the tubercle bacillus.